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(54) **Composition containing lactic acid bacteria for preventing dental caries.**

(57) A composition comprising a lactic acid bacteria capable of staying in the oral cavity and of producing an enzyme for degrading dental plaque.

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The present invention relates to a composition containing lactic acid bacteria, especially a composition having the properties of preventing d ntal cari s and also regulating an intestinal condition, and to a method producing such a composition.

A lactic acid beverage, a food such as a yogurt and containing lactic acid bacteria, and a medicine such as BIOFERMIN (Trade Mark) containing lactic acid bacteria are already in us and wid ly accepted. Most of these composition contain lactic acid bacteria such as Streptococcus lactis, Streptococcus faecalis, Lactobacillus casei, Lactobacillus acidophilus or Lactobacillus bidfidus. These compositions are characterized by their ability to regulate intestinal conditions making use of the property that lactic acid bacteria tend to remain as a human intestinal flora for a while once ingested.

Since the human digestive organs extend from the mouth to the anus through the stomach and the intestine, to acquire an effect such as dental caries prevention in addition to the regulation of the intestinal tract which can be derived only from enteric bacteria, the bacteria must be capable of multiplying and living in the oral cavity.

With regard to dental caries prevention, Japanese Patent Provisional Publication Nos. 62-25, 63-185381 and 63-301788, discloses the use of Streptococcus sanguis prepared by transformation using a genetic engineering technique for producing dextranase and the glucanase which are able to degrade the insoluble glucan which causes the formation of dental plaque.

The present inventor has found, inter alia, that Streptococcus salivarius, which is a lactic acid bacteria, is capable of staying in a flora of the human oral cavity, is harmless to human beings and is capable of extracellular production of dextranase which will effectively degrade dental plaque.

Therefore according to the invention in one aspect there is provided a composition comprising a lactic acid bacteria capable of staying in the oral cavity and of producing an enzyme for degrading dental plaque. The invention extends to a composition, particularly for oral ingestion, which prevents dental caries together with the conventional property of "regulating the intestinal condition". The composition can be a food, a medicine or the like and contains a lactic acid bacteria capable of staying in a flora of the human oral cavity and producing an enzyme for degrading the dental plaque. This bacteria can be combined with other conventional lactic acid bacteria.

There are various bacteria which are indigenous to the oral cavity amongst which the following bacterial species are classified as lactic acid bacteria (Bergey's Manual of Systematic Bacteriology vol.2 ; Williams & Wilkins, 1986, Baltimore, London, Los Angles, Sydney):

Streptococcus salivarius,
Streptococcus sanguis,
Streptococcus mitior,
Streptococcus milleri,
Streptococcus mutans,
Streptococcus rattus,
Streptococcus cricetus,
Streptococcus sobrinus,
Streptococcus ferus,
Streptococcus oralis,
Streptococcus mitis.

breaking down plaque

Among the bacteria listed above, Streptococcus salivarius is harmless to the human species and does not produce insoluble glucan which causes formation of dental plaque.

Streptococcus salivarius is a bacteria which produces a soluble fructan (levan) and, according to the species of the strain, produces an insoluble glucan (dextrans).

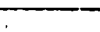
Therefore, strains of Streptococcus salivarius, which produce the dextranase and which are able to degrade insoluble glucan and do not produce insoluble glucan at all, are useful for preventing dental caries.

In order to locate such strains from those available from the principal Strain Preservation Institution of Japan, ten strains of Streptococcus salivarius were selected and examined with respect to their production of dextranase and insoluble glucan.

Example 1: Examination of Dextranase Activity of Streptococcus salivarius

The ten strains of Streptococcus salivarius were each inoculated on to Mitis-salivarius Agar (Difco) to which had been added 1% Chapman Solution thereto, and the shape of colony formed was examined. The results are listed below in Table 1.

Table 1

Strain	Dextranase Activity (*)	Shape of the Colony (**) 30hours culture	48hours culture
M-06	+		
M-17	—		
M-33 (FERM BP-3885)	+++		
G8326	+++		
13956	+		
HHT	±		
HT9R	±		
HT19	∓		
HT32	+		
HT59	∓		

* Dextranase Activity was determined by the size of the Halo produced on the Todd Hewitt Broth (Difco) Agar Plate having been added 0.2% Blue Dextran thereto.

** Shape of the Colony was shown as a sectional (side) view of the Colony produced on the Mitis-salivarius Agar (Difco) Plate having been added 1% Chapman Solution thereto.

The ten strains were then inoculated on to Todd Hewitt Broth (Difco) to which had been added 0.2% Blue Dextran and the approximate potency of the dextranase productivity based on the size of the transparent halo formed two days later of the inoculation determined. Although the conventional knowledge on the dextranase productivity of Streptococcus salivarius was unclear, the results shown in Table 1 indicate that the dextranas productivity of Streptococcus salivarius is different for different strains.

As disclosed in Table 1, Streptococcus salivarius M-33 (FERM BP-3885) and Streptococcus salivarius G8326 produce dextranase remarkably whilst on the other hand Streptococcus salivarius M-17 did not produc any dextranase. Other strains produce some dextranase, but their enzymatic activity was weak.

Streptococcus salivarius M-33 has remarkable dextranase productivity and th potency th reof, judged from the diameter of the halo, is the same level of th Streptococcus sanguis (pMNK-4) construct d by g netic engineering techniques and used as a control. Since the level of dextranase production of Streptococcus salivarius is substantially identical with that of the control, Streptococcus salivarius M-33 is useful for degrading

dental plaque and was, therefore, employed in the following experiments.

Streptococcus salivarius M-33 has been deposited in Fermentation Research Institut , of 1-3, Higashi 1 chome, Tsukuba-shi, Ibaraki-ken 305, Japan., an International Depositary Authority, on the 5th June 1992 under Numb r P-12328.

Exampl 2: Examination on the Degradation Ability of Dental Plaqu by Streptococcus salivarius

Since Streptococcus salivarius M-33 produced remarkable amounts of dextranase, as r ferred to the above, an experiment for eliminating the dental plaque by Streptococcus salivarius M-33 was conducted according to the method shown in the accompanying drawing.

First of all, Streptococcus sobrinus 6715 which is a cariogenic bacteria was pre-cultured on Brain Heart Infusion (Difco) medium at 37°C for 18 hours. A 50µl pre-culture was inoculated into 3ml of Brain Heart Infusion medium supplemented with 1% sucrose. As shown in the drawing, the culture was conducted at 37°C by a static culture wherein 13 x 100 mm test tube (Corning) is sloped 30 degrees. After 18 hours, the amount of the insoluble glucan (dental plaque) adhered to the test tube wall was read in accordance with the order of A, B, C and D of the drawing. The data in the following Table 2 are determined spectrophotometrically as the density for the amount of insoluble glucan at 550nm.

TABLE 2

Culture System	(1)	(2)	(3)	(4)	Total (5)	(3)/(5) (loose)	(4)/(5) (firm)	[(3)+(4)](***)
Mono-culture of 6715	0	0.0108	0.257	0.734	1.002	24.90	74.04	5
co-culture of 6715 and M-33	0.26	0.27	0.91	0.50	1.93	47.36	25.72	73.08
co-culture of 6715 and G8326	0.36	0.18	0.54	0.72	1.80	30.02	40.61	70.63

[***] Total amount of the Dental Plaque

The insoluble glucan of columns 1 and 2 of Table 2 were obtained by "rinse" and are not considered as dental plaque. The insoluble glucan of the column 3 is the fragile dental plaque, obtained by "vortex" and referred to as "loose". The insoluble glucan of the column 4 is form dental plaque obtained by sonication (1 min) and referred to as "firm"

The results shown in Table 2 which indicate the amount of dental plaque formed in the experiment of the mono-culture of Streptococcus sobrinus 6715 (cariogenic bacteria) shows that the most of the dental plaqu produced thereby is "firm".

The examination with regard to the co-culture of Streptococcus sobrinus 6725 and Streptococcus salivarius M-33 was conducted under the same conditions as the control, by culturing and treating a medium inoculated with 50µl if Streptococcus salivarius M-33 and 50µl of Streptococcus sobrinus 6715. As shown in Table 2, th dental plaque obtained in this case contained much dental plaque obtained by "rinse" and "loose" dental plaque and, on the other hand, contained less "firm" dental plaque. A similar tendency is observed in the co-culture of Streptococcus salivarius G8326 and Streptococcus sobrinus 6715 of cariogenic bacteria.

From the results aforementioned, it was found that the co-culture of bacteria which cause the dental caries and Streptococcus salivarius M-33 or Streptococcus salivarius G8326 which both produce dextranase, reduces "firm" dental plaque and increases "loose" plaque. In other words, in the case of the co-culture of a bacteria which causes the dental caries and Streptococcus salivarius G8326, although dental plaque is produced, the fragile and removable glucan, wherein the alpha-1,6 bond thereof constitutes dental plaque are broken by the dextranase activity, would seem to be increased.

Example 3: The Relation between th D gree of th Dextranase Productivity of Streptococcus salivarius and the Shape of the Colony

In view of the results of Examples 1 and 2, degradation ffect of dental plaque by the strains Streptococcus

salivarius having high dextranase productivity was confirmed. Therefore an experiment to specify the subgroup of Streptococcus salivarius having the f atur s aforementioned was conducted.

Each strain of Streptococcus salivarius as used in Example 1 was modulated onto Mitis-Salivarius Agar Plate (Difco), which had been added 1% Chapman solution and the plates w re then incubated at 37°C. After about 30 hours incubation, most of the strains had grown to the typical large smooth colony as disclosed in the right column of Table 1. After 48 hours incubation of continuous cultivation, the center of the colony of Streptococcus salivarius M-33 or Streptococcus salivarius G826 had sunk and the colony gradually formed into a so called crater-form. In stains having weak dextranase productivity, such phenomenom was not observed. In contrast thereto, the strain having no dextranase productivity formed an untypical rough colony.

Accordingly, a correlation between high dextranase productivity and the shape of the colony, i.e. those what produce dextranase give a greater from after 48 hours or more incubation.

The correlation between the degree of the dextranase productivity and the shape of the colony can be understood easily for the following reasons. Generally, Streptococcus salivarius produce, as an extracellular polysaccharide, the water-soluble fructan (levan) and the water-insoluble glucan (dextran). Since the polysaccharide was produced according to cell multiplication, the colony thereof would be formed into the raised, glossy smooth colony. Since the dextranase productivity of the most of Streptococcus salivarius strains are weak as shown in the left column of Table 1, the dextrans would not be degraded by the prolonged cultivation and the shape of the colony would be unchanged. In contrast thereto, although Streptococcus salivarius M-33 or Streptococcus salivarius G8326 produce smooth colony at the early stage, prolonged cultivation would form a crater shaped colony because the dextran was degraded in site of cell multiplication stopping.

The present invention provides a composition, such as a food or medicine, containing lactic acid bacteria which the composition has the novel property of "dental caries prevention" together with the conventional property of "regulating the intestinal condition".

Claims

1. A composition comprising a lactic acid bacteria capable of staying in the oral cavity and of producing an enzyme for degrading plaque.

2. A composition as claimed in Claim 1 in which the lactic acid bacteria is a Streptococcus and the enzyme is dextranase.

3. A composition as claimed in Claim 2 in which the bacteria is Streptococcus salivarius and produces a crater-form colony.

4. A composition as claimed in Claim 2 in which the bacteria is Steptococcus salivarius M-33 (FERM BP-3885).

5. A lactic acid bacteria capable of staying in the oral cavity and of producing an enzyme for degrading dental plaque for use in preventing dental caries.

6. A lactic acid Streptococcus salivarius bacteria capable of multiplying and living in the oral cavity and of producing dextranase for use in preventing dental caries.

7. Streptococcus salivarius M-33 for use in preventing dental caries.

8. The use of a lactic acid bacteria capable of staying in the oral cavity and of producing an enzyme for degrading dental plaque for the manufacture of a composition for oral ingestion for the treatment of dental plaque.

9. The use of Streptococcus salivarius M-33 for the manufacture of a composition for oral ingestion for the treatment of dental plaque.

10. A method for producing the composition comprising a lactic acid bacteria capable of staying in the oral cavity and producing an enzyme for degrading the dental plaque, comprising:
    (a) culturing, in a medium, a lactic acid bact ria capable of staying in the oral cavity and producing an enzyme for degrading the d ntal plaque,
    (b) selecting th strain producing a crater-form colony,

(c) multiplying the s lected strain, and
(d) preparing th composition by incorporating the multipli d strain therein.